EXHIBIT 14

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Talcum powder, chronic pelvic inflammation and NSAIDs in relation to risk of epithelial ovarian cancer

Melissa A. Merritt[1,2], Adèle C. Green[1], Christina M. Nagle[1], Penelope M. Webb[1]*, Australian Cancer Study (Ovarian Cancer) and Australian Ovarian Cancer Study Group

[1]*Population Studies and Human Genetics Division, Queensland Institute of Medical Research, Brisbane, Queensland, Australia*
[2]*School of Population Health, University of Queensland, Brisbane, Queensland, Australia*

Chronic inflammation has been proposed as the possible causal mechanism that explains the observed association between certain risk factors, such as the use of talcum powder (talc) in the pelvic region and epithelial ovarian cancer. To address this issue we evaluated the potential role of chronic local ovarian inflammation in the development of the major subtypes of epithelial ovarian cancer. Factors potentially linked to ovarian inflammation were examined in an Australia-wide case–control study comprising 1,576 women with invasive and low malignant potential (LMP) ovarian tumours and 1,509 population-based controls. We confirmed a statistically significant increase in ovarian cancer risk associated with use of talc in the pelvic region (adjusted odds ratio 1.17, 95% CI: 1.01–1.36) that was strongest for the serous and endometrioid subtypes although the latter was not statistically significant (adjusted odds ratios 1.21, 95% CI 1.03–1.44 and 1.18, 95% CI 0.81–1.70, respectively). Other factors potentially associated with ovarian inflammation (pelvic inflammatory disease, human papilloma virus infection and mumps) were not associated with risk but, like others, we found an increased risk of endometrioid and clear cell ovarian cancer only among women with a history of endometriosis. Regular use of aspirin and other nonsteroidal anti-inflammatory drugs was inversely associated with risk of LMP mucinous ovarian tumours only. We conclude that on balance chronic inflammation does not play a major role in the development of ovarian cancer.

Key words: ovarian cancer; chronic inflammation; talcum powder

Chronic inflammation (hereafter referred to as inflammation) was first invoked as a possible mechanism leading to the development of epithelial ovarian cancer to explain observed associations between certain factors, such as use of talcum powder in the perineal region or pelvic inflammatory disease (PID) and risk of ovarian cancer.[1] The major mechanisms thought to underlie ovarian carcinogenesis, namely increased pituitary gonadotropins or incessant ovulation, do not explain such associations.

A link between inflammation and cancer in general has long been recognized. As early as 1863, Virchow noticed the presence of leukocytes in cancer tissues and suggested a possible connection between inflammation and cancer.[2] Since inflammation also represents the process by which the immune system responds to infection or irritation, however, it has been referred to as a 'double-edged sword' with acute (beneficial) inflammation distinguished from the chronic (detrimental) inflammation that may prevent a robust anti-tumour response.[3]

Indeed the most consistent evidence linking inflammation with ovarian cancer comes from the many reports that use of talc in the perineal region increases ovarian cancer risk.[4,5] It has been suggested that the association between talc use and ovarian cancer is strongest for serous tumours when compared to other less common subtypes.[4,6,7] This would be consistent with the histological similarities observed between serous ovarian cancer and mesothelioma, which is known to be caused by asbestos, and the shared

Abbreviations: ACS, Australian Cancer Study; AOCS, Australian Ovarian Cancer Study; BMI, body mass index; HPV, human papilloma virus; LMP, low malignant potential; NSAIDs, non-steroidal anti-inflammatory drugs; OC, oral contraceptive; PID, pelvic inflammatory disease; STI, sexually transmitted infection.

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***Correspondence to:** Queensland Institute of Medical Research, PO Royal Brisbane and Women's Hospital, Brisbane, Queensland 4029, Australia. Fax: +61-7-3845-3502. E-mail: penny.webb@qimr.edu.au

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chemical properties of talcum powder and asbestos. Testing various factors that are possibly related to ovarian inflammation in a case–control study, Ness *et al.*[8] found that perineal talc use and endometriosis, defined as the presence of endometrial tissue outside the uterus and associated with localised inflammation at the site of endometriotic implants, were positively associated with ovarian cancer risk. However, they saw no association with PID, which they had also expected to be associated with increased risk.[8] Extending these epidemiological analyses, McSorley *et al.*[9] recently found significantly higher circulating C-reactive protein (CRP) levels, a marker of systemic chronic inflammation, among 167 women with incident ovarian cancer risk in a multicentre nested case–control study.

The potential role of ovarian inflammation in the development of ovarian cancer remains an open question. The aim of the current study was to further examine the role of local chronic inflammation in the development of epithelial ovarian cancer overall and by histologic subtype. In addition to talcum powder use, we examined medical conditions that cause inflammation in the pelvic region, including endometriosis and PID, and we also tested the hypothesis that if inflammation causes ovarian cancer then regular use of anti-inflammatory drugs should be inversely associated with this disease.

Material and methods

Study design

The Australian Ovarian Cancer Study is an Australia-wide population-based case–control study of epithelial ovarian cancer. It includes incident cases of invasive and low malignant potential (LMP) ovarian cancer diagnosed in women (aged 18–79 years) between January 2002 and June 2005. A total of 3,553 women were identified with suspected ovarian cancer. Of these, 304 died before contact could be made, physicians refused to give consent to contact 133, usually because they were too sick or unable to give informed consent and 194 women could not be contacted. A further 167 (5%) were excluded on the basis of language difficulties (70), mental incapacity (33) and illness (64). The remaining 2,755 women were invited to participate and, of these, 2,319 (84% of those approached) agreed to take part.

Two researchers independently abstracted information on tumour site, histological subtype and tumour behaviour (invasive *vs.* LMP) from the diagnostic histopathology reports and discrepancies were resolved by consensus. For a sample of 87 women, the pathology reports and full set of diagnostic slides were reviewed by a gynaecologic pathologist and the agreement with the original abstracted data was more than 97% for tumour site, behaviour and subtype. After histopathology review, 624 women were excluded because they were found to have nonepithelial, nonovarian or benign tumours and 10 because their cancer was first diagnosed before the start of the study period. Of the final 1,685 eligible participants with invasive or LMP cancers of the ovary, peritoneum or fallopian tube, 1,576 (94%) returned a questionnaire and comprised the case population in the current study. Separate analyses were also carried out for the 994 serous, 191 mucinous, 141 endometrioid and 88 clear cell tumours (the remaining 162 tumours were of other epithelial or mixed subtypes).

Potential control participants were identified from the Australian Electoral Roll (all citizens are required by law to enrol). Controls were frequency-matched to the entire case series based on age (5-year groups) and state of residence. In all, 3,600 women were contacted. Of these, 158 were ineligible because of language difficulties ($n = 97$) or illness ($n = 61$) and 16 were unable to be contacted a second time. Of the 3,426 eligible women, 1,612 (47%) agreed to participate and returned a questionnaire. From these women, 6 were excluded because they reported a previous ovarian cancer and 97 because of a previous bilateral oophorectomy resulting in a total of 1,509 controls for study.

Study participants filled in a comprehensive health and lifestyle questionnaire, which included questions about their personal details, physical characteristics, family history, medical and surgical history, lifestyle habits and reproductive factors. To determine use of talcum powder in the perineal region, participants were asked whether they had ever used powder or talc in the genital area or on underwear or sanitary pads/diaphragm. They were asked their age at first use and years of talc use in these areas. Duration of talcum powder use prior to and after hysterectomy/tubal ligation was calculated and in all analyses perineal talc use was defined as use occurring while the reproductive tract was patent (*i.e.*, prior to hysterectomy/tubal ligation for those women who had undergone gynaecological surgery). Information on talc use under the arms or on the chest or abdomen was also collected.

To measure use of nonprescription anti-inflammatory medications, participants were given examples of the type of medication (*e.g.*, aspirin) followed by a list of the common generic and brand names. To quantify the frequency of use, participants were asked how often they had taken various medications over the past 5 years (ranging from never to as much as twice or more per day). The current analyses were restricted to medications known to suppress inflammation namely aspirin and other nonsteroidal anti-inflammatory drugs (NSAIDs). Participants were also asked whether they had ever had any of a number of specific medical conditions and, if so, the ages at which these were diagnosed.

Ethics approval was received from the Human Research Ethics Committees at the Queensland Institute of Medical Research, Peter MacCallum Cancer Centre, University of Melbourne, all participating hospitals and cancer registries.

Statistical analysis

Risk estimates were calculated as odds ratios (OR) with 95% confidence intervals (CI). χ^2-Squared tests were used to test for differences in patient characteristics (*e.g.*, age, level of education). All significance tests were 2-sided and a p-value of less than 0.05 was taken as significant. Unconditional multiple logistic regression models were constructed to simultaneously adjust for confounding factors.

Exposures to factors of interest occurring in the 12 months prior to diagnosis for cases (or 12 months prior to first contact for controls) were excluded because the aetiological influence of very recent exposures on incident ovarian cancer is likely to be minimal and, in cases, recent behaviours may reflect the presence of subclinical disease. All models were adjusted for the categorical variables of age in 10-year groups (<50, 50–59, 60–69, ≥ 70), highest level of education, parity (number of pregnancies >6 months) and duration of contraceptive use (including oral contraceptive pills and contraceptive injections). Analyses of endometriosis and potential symptoms of endometriosis (painful or long periods) were also adjusted for the categorical variable of body mass index (BMI) 1 year prior to diagnosis/recruitment (≤ 24.9, 25–29.9, ≥ 30 kg/m^2). Other potential confounders that were considered for all analyses but not included in the final models since they did not substantially alter risk estimates were: income, family history of ovarian or breast cancer, hysterectomy and/or tubal ligation and smoking.

All analyses were performed using the SAS system V 9.1 (SAS Institute, Cary, NC). Tests for linear trend were performed using the maximum likelihood test with the categorical variable of interest entered as a continuous term.

Results

The final study population included 1,576 women with epithelial ovarian cancer (invasive and LMP) and 1,509 controls. Cases were significantly older than controls (mean age cases = 57.8, mean age controls = 56.42, $p = 0.001$) and were less likely to have continued their education beyond high school (Table I). As expected, cases were significantly more likely to be nulliparous

TABLE I – DESCRIPTIVE CHARACTERISTICS OF 1,576 WOMEN WITH EPITHELIAL OVARIAN CANCER AND 1,509 RANDOMLY SELECTED POPULATION-BASED CONTROLS

Variable	Controls[1] (*N* = 1,509) *N* (%)	Cases[1] (*N* = 1,576) *N* (%)	*p*-Value
Highest level of education			
High school	735 (49)	851 (54)	0.02[2]
Technical college/ trade certificate	550 (37)	502 (32)	
University	218 (15)	214 (14)	
Number pregnancies (≥6 months)			
Nulliparous	181 (12)	298 (19)	<0.0001[3]
1–2	644 (43)	647 (41)	
≥3	684 (45)	628 (40)	
Ever used oral contraceptives			
No	330 (22)	505 (32)	<0.0001[3]
≤5 years	361 (24)	432 (28)	
>5 years	811 (54)	619 (40)	
Previous tubal ligation	406 (27)	355 (23)	0.0003[2]
Previous hysterectomy	289 (19)	364 (23)	0.05[2]
Mother/sister with ovarian or breast cancer	195 (13)	273 (19)	0.002[2]

[1]Numbers may not sum to total because of missing data.–[2]χ^2-square test for heterogeneity, adjusted for age group (10 year categories).–[3]χ^2-square test for trend, adjusted for age group (10 year categories).

and to report a mother or sister with ovarian or breast cancer. Cases were less likely to have used oral contraceptives or to report a previous tubal ligation. Unexpectedly, cases were somewhat more likely to report a prior hysterectomy (Table I).

Ever use of talc in the perineal region (among women with patent fallopian tubes) was associated with a significant increase in risk of all types of epithelial ovarian cancer combined (adjusted OR = 1.17, 95% CI: 1.01–1.36) (Table II). Analysis by histological subtype showed that the increase in risk was strongest for serous and endometrioid tumours although it was only statistically significant for serous tumours (adjusted OR = 1.21, 95% CI: 1.03–1.44 and 1.18, 95% CI 0.81–1.70, respectively). This increased risk was seen for both invasive and LMP serous tumours (data not shown), although the association with LMP tumours was not statistically significant because of the smaller numbers. There was no clear trend of increasing risk with longer duration of use, although tests for trend were of borderline statistical significance for all cancers and the serous subgroup ($p = 0.02$ for both). When we considered invasive and LMP tumours separately, a modest but statistically significant increase in risk of invasive serous tumours was observed in the highest category of use (over 25 years, adjusted OR = 1.35, 95% CI: 1.06–1.72), whereas little or no increased risk was observed with less than 25 years of use. For serous LMP tumours, a modest increase in risk was observed only in the lowest duration of use category (upto 10 years, adjusted OR = 1.71, 95% CI: 1.07–2.73) with no association for over 10 years of use.

Increased risk of ovarian cancer was specifically related to talc use in the pelvic region as talc use on other body sites showed no association (OR = 1.01, 95% CI: 0.84–1.20). In contrast to the elevated risk of ovarian cancer observed with perineal talc use prior to hysterectomy and/or tubal ligation, talc use after such surgery showed no association with serous ovarian cancer risk, regardless of duration (Table II).

Prior to 1976, talcum powder was often contaminated with asbestos fibres.[10,11] To assess whether the association between use of talc and ovarian cancer risk varied over time we evaluated this separately for different age groups. Our assumption was that use of talcum powder among older women would largely have been prior to 1976 (when voluntary guidelines to prevent asbestos contamination of talcum powder were adopted) whereas a greater pro-

TABLE II – ASSOCIATION BETWEEN PERINEAL TALCUM POWDER USE (SEPARATING THE EFFECTS OF USE PRIOR TO AND AFTER HYSTERECTOMY AND/OR TUBAL LIGATION) AND RISK OF EPITHELIAL OVARIAN CANCER

	Controls[1] *N* (%)	All cases[1] *N* (%)	All cases (*N* = 1,576) OR[2] (95% CI)	Serous (*N* = 994) OR[2] (95% CI)	Mucinous (*N* = 191) OR[2] (95% CI)	Endometrioid (*N* = 141) OR[2] (95% CI)	Clear cell (*N* = 88) OR[2] (95% CI)
Perineal use of talcum powder[3]							
Never	835 (57)	821 (54)	1.0	1.0	1.0	1.0	1.0
Ever	635 (43)	702 (46)	1.17 (1.01–1.36)	1.21 (1.03–1.44)	1.10 (0.80–1.52)	1.18 (0.81–1.70)	1.08 (0.68–1.72)
Use pre- or no-surgery[3]							
None	835 (57)	821 (54)	1.0	1.0	1.0	1.0	1.0
>0–10 years	193 (13)	200 (13)	1.13 (0.90–1.41)	1.26 (0.98–1.63)	0.79 (0.47–1.33)	1.05 (0.59–1.85)	1.08 (0.52–2.27)
>10–25 years	214 (15)	213 (14)	1.08 (0.87–1.34)	1.03 (0.80–1.32)	1.34 (0.86–2.08)	1.14 (0.67–1.94)	0.96 (0.48–1.90)
>25 years	228 (16)	289 (19)	1.29 (1.04–1.58)	1.34 (1.06–1.68)	1.21 (0.75–1.97)	1.31 (0.80–2.16)	1.18 (0.63–2.22)
p-Value (trend)			*0.021*	*0.022*	*0.27*	*0.28*	*0.69*
Use post-surgery							
None	1,294 (88)	1,340 (88)	1.0	1.0	1.0	1.0	1.0
>0–10 years	49 (3)	50 (3)	1.08 (0.71–1.62)	1.07 (0.67–1.69)	1.39 (0.60–3.19)	0.97 (0.34–2.77)	0.64 (0.15–2.81)
>10–25 years	81 (6)	87 (6)	1.14 (0.82–1.57)	1.03 (0.72–1.48)	2.04 (1.09–3.79)	1.03 (0.45–2.32)	0.44 (0.11–1.88)
>25 years	46 (3)	46 (3)	1.00 (0.64–1.51)	1.09 (0.69–1.71)	0.91 (0.27–3.05)	0.79 (0.23–2.64)	0.43 (0.06–3.22)
p-Value (trend)			*0.61*	*0.60*	*0.12*	*0.81*	*0.16*
Ever[3] vs. never use stratified by age at diagnosis/recruitment							
<50 years	143 (23)	137 (20)	1.16 (0.86–1.57)	1.53 (1.06–2.19)	1.42 (0.89–2.25)	0.66 (0.28–1.55)	0.98 (0.41–2.29)
50–59 years	213 (33)	237 (34)	1.22 (0.93–1.59)	1.20 (0.89–1.62)	0.76 (0.46–1.26)	1.41 (0.78–2.54)	1.67 (0.88–3.15)
60–69 years	191 (30)	207 (29)	0.93 (0.70–1.23)	0.95 (0.70–1.29)	0.83 (0.49–1.40)	1.31 (0.62–2.75)	0.87 (0.40–1.85)
≥70 years	88 (14)	121 (17)	1.61 (1.10–2.36)	1.66 (1.08–2.56)	0.91 (0.42–1.97)	1.32 (0.50–3.49)	1.41 (0.58–3.35)

[1]Numbers may not sum to total because of missing data.–[2]Adjusted for age (except age-stratified analysis), education, parity and oral contraceptive pill use.–[3]Analysis restricted to use while the genital tract was unobstructed (i.e., prior to hysterectomy).

portion of use in younger women would have been after that date. Significantly elevated risks of ovarian cancer overall and for the serous subtype were seen in women who were 70 years of age or older and also among those who were less than 50 for the serous subtype only. A modest increase in risk was also observed in the 50–59 year group (nonsignificant) however no association was observed in the 60–69 year age group. Similar results were observed when invasive tumours were examined separately (the number of LMP tumours was too small to evaluate the effects by age).

Table III shows no significant association was observed between PID and risk of all subtypes of ovarian cancer combined (OR = 1.15, 95% CI: 0.85–1.57), or for the different histological subtypes. When we examined the association relative to the time elapsed since diagnosis of PID, no association with ovarian cancer risk was observed (data not shown).

A reported history of genital herpes was not associated with risk of all subtypes of ovarian cancer combined (OR = 1.17, 95% CI: 0.73–1.87). However, a significant positive association was seen with risk of serous tumours (OR = 1.65, 95% CI: 1.01–2.69; Table III), with similar nonsignificant increases observed for both invasive (OR = 1.65, 95% CI: 0.98–2.78) and LMP serous tumours (OR = 1.76, 95% CI: 0.71–4.34). For serous tumours, similar increased risks were seen for both more recent (less than 20 years) and long-term (over 20 years) infection (data not shown).

Neither HPV infection, based on self-reported history of abnormal pap smears and/or genital warts, nor a history of mumps after the age of puberty were associated with risk of ovarian cancer overall (Table III). There was also no association with mumps when we considered infection at any age (OR = 0.95, 95% CI: 0.81–1.12). There was however a suggestion that HPV infection was associated with a slightly increased risk of the endometrioid subtype (OR = 1.58, 95% CI: 1.03–2.44). Analyses considering time since the condition was first reported did not alter these results.

We found no significant association between a reported history of endometriosis and ovarian cancer risk overall (OR = 1.31, 95% CI: 0.97–1.78). However statistically significant increased risks were seen for the endometrioid and clear cell subtypes (OR = 1.85, CI: 1.02–3.38 and OR = 2.66, CI: 1.31–5.44, respectively). Because endometriosis may go undiagnosed, we also considered a reported history of potential symptoms of endometriosis (long or painful periods) however neither was associated with ovarian cancer risk (Table III). Similar results were noted when the analysis was restricted to women who had not used hormonal contraceptives. As with other medical conditions, risk estimates did not vary with time elapsed since endometriosis was first reported.

For comparison with inflammation believed to occur in close proximity to the ovaries, medical conditions associated with inflammation at other body sites were also examined (including gall stones, inflammatory bowel disease, diverticulitis, oesophagitis, gastritis and pancreatitis). None of these conditions was associated with ovarian cancer risk (data not shown).

To assess whether regular use of anti-inflammatory medications was inversely associated with ovarian cancer risk, use of aspirin and NSAIDs in the 5 years prior to study recruitment was examined. Any use of aspirin was not associated with ovarian cancer risk for all subtypes combined (OR for any *vs.* no use = 1.06, 95% CI: 0.92–1.23; Table IV) or for any of the individual subtypes. Ever use of NSAIDs in the last 5 years also had no effect on risk of all subtypes of ovarian cancer (OR = 0.88, 95% CI: 0.76–1.02). However, risk of mucinous tumours was inversely associated with any use of NSAIDs (OR = 0.69, 95% CI: 0.50–0.94) and a further decrease in risk was observed with more frequent use (p-value trend = 0.01). Separate analyses of invasive (n = 44) and LMP (n = 147) mucinous tumours demonstrated that the observed inverse association was driven entirely by LMP tumours (OR for any *vs.* no use = 0.59, 95% CI: 0.41–0.84, compared to

TABLE III – ASSOCIATION BETWEEN SELF-REPORTED MEDICAL CONDITIONS POTENTIALLY ASSOCIATED WITH INFLAMMATION OF THE OVARIES AND RISK OF EPITHELIAL OVARIAN CANCER

	Controls[1] *N* (%)	All cases[1] *N* (%)	All cases (*N* = 1,576) OR[2] (95% CI)	Serous (*N* = 994) OR[2] (95% CI)	Mucinous (*N* = 191) OR[2] (95% CI)	Endometrioid (*N* = 141) OR[2] (95% CI)	Clear cell (*N* = 88) OR[2] (95% CI)
PID							
Never	1,406 (94)	1,460 (93)	1.0	1.0	1.0	1.0	1.0
Ever	84 (6)	103 (7)	1.15 (0.85–1.57)	0.96 (0.66–1.38)	1.46 (0.82–2.60)	1.29 (0.66–2.52)	0.87 (0.30–2.49)
Genital herpes							
Never	1,420 (98)	1,425 (97)	1.0	1.0	1.0	1.0	1.0
Ever	35 (2)	42 (3)	1.17 (0.73–1.87)	1.65 (1.01–2.69)	0.40 (0.09–1.71)	0.32 (0.04–2.37)	0.74 (0.10–5.63)
HPV infection							
Never	1,148 (78)	1,197 (81)	1.0	1.0	1.0	1.0	1.0
Ever	317 (22)	273 (19)	0.94 (0.78–1.15)	0.92 (0.74–1.15)	0.98 (0.66–1.45)	1.58 (1.03–2.44)	0.72 (0.36–1.47)
Mumps							
Never	496 (76)	508 (75)	1.0	1.0	1.0	1.0	1.0
Ever (postpubertal)	160 (24)	164 (25)	0.96 (0.73–1.25)	1.06 (0.79–1.42)	0.78 (0.40–1.49)	0.97 (0.50–1.87)	0.81 (0.35–1.92)
Endometriosis[3]							
Never	1,413 (94)	1,431 (92)	1.0	1.0	1.0	1.0	1.0
Ever	87 (6)	124 (8)	1.31 (0.97–1.78)	1.14 (0.80–1.62)	0.89 (0.46–1.75)	1.85 (1.02–3.38)	2.66 (1.31–5.44)
Long periods[3] (>7 days)							
Never/rarely	1,174 (82)	1,173 (82)	1.0	1.0	1.0	1.0	1.0
Often	188 (13)	192 (14)	1.05 (0.83–1.31)	1.05 (0.81–1.36)	0.70 (0.40–1.22)	1.23 (0.71–2.12)	1.26 (0.62–2.53)
Always	75 (5)	62 (4)	0.79 (0.55–1.13)	0.82 (0.55–1.23)	0.78 (0.34–1.78)	0.72 (0.27–1.85)	0.83 (0.24–2.83)
Painful periods[3]							
Never/rarely	760 (52)	711 (49)	1.0	1.0	1.0	1.0	1.0
Sometimes	290 (20)	301 (20)	1.04 (0.85–1.27)	1.04 (0.83–1.31)	0.95 (0.61–1.47)	1.07 (0.65–1.75)	1.13 (0.59–2.15)
Often	404 (28)	452 (31)	1.17 (0.98–1.40)	1.17 (0.96–1.43)	1.12 (0.77–1.64)	1.12 (0.72–1.73)	1.14 (0.65–2.00)

[1]Numbers may not sum to total because of missing data.–[2]Adjusted for age, education, parity and oral contraceptive pill use.–[3]Additionally adjusted for body mass index one year prior to diagnosis.

TABLE IV – ASSOCIATION BETWEEN ANTI-INFLAMMATORY MEDICATION USE IN THE PAST 5 YEARS AND RISK OF EPITHELIAL OVARIAN CANCER

	Controls[1] *N* (%)	All cases[1] *N* (%)	All cases (*N* = 1,576) OR[2] (95% CI)	Serous (*N* = 994) OR[2] (95% CI)	Mucinous (*N* = 191) OR[2] (95% CI)	Endometrioid (*N* = 141) OR[2] (95% CI)	Clear cell (*N* = 88) OR[2] (95% CI)
Aspirin							
Never	772 (51)	783 (50)	1.0	1.0	1.0	1.0	1.0
Ever	730 (49)	781 (49)	1.06 (0.92–1.23)	1.06 (0.90–1.25)	0.99 (0.72–1.35)	0.92 (0.64–1.32)	0.92 (0.58–1.45)
≤1/week	612 (41)	650 (41)	1.06 (0.91–1.23)	1.05 (0.88–1.25)	0.98 (0.71–1.36)	0.98 (0.68–1.43)	0.95 (0.59–1.54)
≥2/week	118 (8)	131 (8)	1.06 (0.80–1.41)	1.11 (0.81–1.51)	1.02 (0.52–2.03)	0.56 (0.23–1.34)	0.75 (0.30–1.89)
p-Value (trend)			*0.5*	*0.4*	*0.99*	*0.4*	*0.6*
NSAIDs							
Never	625 (42)	723 (46)	1.0	1.0	1.0	1.0	1.0
Ever	878 (58)	836 (54)	0.88 (0.76–1.02)	0.93 (0.78–1.10)	0.69 (0.50–0.94)	0.76 (0.53–1.09)	0.92 (0.58–1.45)
≤1/week	653 (43)	625 (40)	0.90 (0.76–1.05)	0.94 (0.78–1.12)	0.73 (0.53–1.02)	0.73 (0.50–1.09)	0.97 (0.59–1.60)
≥2/week	225 (15)	211 (14)	0.83 (0.66–1.04)	0.90 (0.70–1.16)	0.51 (0.28–0.93)	0.84 (0.49–1.44)	0.79 (0.39–1.58)
p-Value (trend)			*0.1*	*0.3*	*0.01*	*0.3*	*0.6*

[1]Numbers may not sum to total because of missing data.–[2]Adjusted for age, education, parity and oral contraceptive pill use.

1.17, 95% CI 0.62–2.21 for invasive mucinous tumours). There was also a dose-response relationship for LMP mucinous tumours (OR for 2 or more pills per week *vs.* no use = 0.46, 95% CI: 0.23–0.91, *p*-value trend = 0.01).

Discussion

The hypothesis that chronic inflammation may lead to the development of epithelial ovarian cancer was first proposed to explain how certain factors, such as talc use in the perineal region, may be linked to increased risk of developing ovarian cancer.[1] Testing the inflammation hypothesis in a case–control study, Ness *et al*. found that proinflammatory factors, such as perineal talc use and endometriosis increased ovarian cancer risk, but others such as PID did not significantly increase ovarian cancer risk (separate analyses of individual histological subtypes of ovarian cancer were not presented).[8] Consistent with this hypothesis, McSorley *et al*.[9] recently reported a trend of increasing ovarian cancer risk with increasing levels of CRP, a marker of inflammation. However, given the lack of specificity of CRP and its association with prevalent chronic conditions, such as ischaemic heart disease,[12] it is difficult to rule out confounding as an alternate explanation for these results.[9] Until the present study, no other epidemiological studies appear to have tested the hypothesis that ovarian inflammation is associated with ovarian cancer risk. In the current study, a significantly elevated risk of ovarian cancer overall and of the serous subtype associated with perineal talc use was identified. A nonsignificant increase in risk was also seen for endometrioid tumours. Other factors that could potentially cause ovarian inflammation (such as PID, HPV infection, mumps and endometriosis) were not associated with ovarian cancer risk overall, however there was some evidence of a positive association with some of these factors in the subtype specific analyses. These results in combination with previous studies suggest that chronic inflammation is unlikely to play a major role in the development of ovarian cancer.

Focusing on talc use, we found that any use of perineal talc was associated with a small but significantly increased risk of ovarian cancer overall and specifically amongst the invasive and LMP serous tumours although no clear dose-response with increasing duration of use was identified. This finding is consistent with results of previous studies.[4,6,7,10,13,14]

As expected, ovarian cancer risk was only related to talc use in women with no surgical closure of the fallopian tubes or those who had used talc presurgery, with no association seen for talc use after tubal sterilisation or hysterectomy. Similar observations were made in previous case–control studies of ovarian cancer (all subtypes) with elevated risks observed in women who had not had a tubal ligation[4,14] or those who had used talc presurgery.[13] These former studies together with the current findings support the hypothesis that talc particles are transported to the ovaries *via* unobstructed fallopian tubes. In contrast, the Nurses' Health study found no increase in risk among women who were perineal talc users but had never had a tubal ligation.[7]

While it has been demonstrated experimentally that talc particles can reach the ovaries in humans and rodents as the result of talc use in the pelvic region,[15–17] ovarian talc particle burden in normal human ovaries is not correlated with reported exposure levels.[17] This suggests that use of only a small amount of talc may be required for some talc to reach the ovaries and increase risk of cancer.

It has been hypothesised that talc is linked to ovarian cancer development through inflammation, however evidence linking an inflammatory response with talc contamination of the ovaries is lacking. Talc-induced inflammation is unlikely to be in the formation of granulomas as these are rarely observed in human ovaries.[18,19] Other likely manifestations of talc-induced inflammation include reduced fibrinolysis, activation of neutrophils and macrophages and increased production of cytokines and growth factors, and these have been suggested to occur in the peritoneum in response to contamination by surgical glove powder.[20] Rigorous investigation of the precise biological response of the ovarian surface epithelium to perineal talc use is needed.

We also sought to determine whether possible contamination of talc with asbestos fibres, which are known to cause inflammation of epithelial tissues, could explain the observed link between perineal talc use and serous ovarian cancer. Voluntary guidelines to prevent asbestos contamination of cosmetic talc were introduced in 1976 and consequently earlier formulations were more likely to contain asbestos fibres.[10,11] Increased risk of serous ovarian cancer was not restricted to perineal talc use in the oldest age groups, who were more likely to have been exposed to asbestos-contaminated talc, but was also observed in the youngest (less than 50 years) and the 50–59 year old age group. Other studies have also reported no increase in risk of all subtypes of ovarian cancer associated with talc use before 1970[13] or before 1975.[14] These findings contrast with 2 other reports of increased risk of serous[7] and all subtypes of epithelial ovarian cancer[10] associated with earlier use of talc.

If inflammation plays a role in the aetiology of ovarian cancer then it would be expected that PID would be associated with increased risk of ovarian cancer. PID was not associated with elevated risk of ovarian tumours in our data, confirming several previous reports of no association with PID in studies of all subtypes of ovarian cancer.[8,21,22] To date there has been only one report of a significant positive association between PID and ovarian cancer.[23] Genital herpes infection was associated with a nonsignificant increased risk of invasive serous cancer in our data, although this observation was based on a small number of exposed cases ($n = 27$). One previous study found no association between genital herpes and ovarian cancer risk (the number of exposed cases was not reported).[8] Latent infection by herpes virus is established

in the nerve root ganglia and it is associated with a variety of initial and recurrent symptoms such as genital ulceration.[24] It is biologically plausible that inflammation associated with genital herpes infection could increase risk of ovarian cancer as Herpes simplex virus type 2 has been detected in the upper genital tract of women with acute PID[25,26] and acute salpingitis.[27] Further studies are needed to confirm this association.

HPV infection (based on reports of abnormal pap smears and/or genital warts) showed no association with ovarian cancer risk, except for the endometrioid subtype. We hypothesised that HPV infection could potentially cause ovarian inflammation as HPV DNA has been identified in the ovaries of patients with primary ovarian squamous intraepithelial neoplasia[28,29] and in the upper genital tract of patients with cervical squamous carcinoma.[30] In addition, high-risk HPV DNA has been reported in 10% of ovarian epithelial carcinomas.[31] Abnormal pap smears and genital warts are generally associated with HPV genotypes classified as high-risk and low-risk, respectively, in regards to their association with carcinogenic transformation.[32] However, separate analyses also showed no association with ovarian cancer risk.

Mumps infection (either after puberty or at any age) was not associated with ovarian cancer risk. It has been estimated that some 5% of postpubertal mumps cases are associated with clinically apparent oophoritis, which in severe cases could result in infertility caused by nonfunctional ovarian tissue.[33] We were unable to identify these particular cases in the current analysis and therefore further study is needed to examine the association between mumps oophoritis and ovarian cancer.

While endometriosis is a condition associated with localised inflammation, it is also related to changes in hormone levels (increased oestrogen unopposed by progesterone) at the site of endometriotic implants.[34] Despite this, endometriosis or potential symptoms of endometriosis (long or painful periods) were not associated with ovarian cancer risk overall, but there was an increased risk of endometrioid and clear cell subtypes among women who reported a history of endometriosis. This result was anticipated because current epidemiological evidence suggests that endometriosis is most strongly associated with the endometrioid and clear cell subtypes of ovarian cancer.[35,36]

Finally, if inflammation did promote epithelial ovarian cancer development, then it may be reasonably expected that regular use of anti-inflammatory medications would reduce risk. However, no overall association with ovarian cancer risk was observed in the current study. This supports results from 2 recent meta-analyses, which have also not shown that regular use of anti-inflammatory medications (aspirin or other NSAIDs) reduces ovarian cancer risk.[37,38] Of interest however was the apparent inverse association between NSAID use and the mucinous subtype, which was entirely driven by the LMP group. We know from other epidemiological studies that the aetiology of mucinous tumours differs in a number of ways from the other subtypes of ovarian cancer, so NSAID use may be another factor to add to this list. However, this result awaits confirmation by others.

Strengths of our study included its large size (1,576 women with ovarian cancer and 1,509 population-based controls) and Australia-wide coverage. A limitation was the low response rate for controls (47%), which could have resulted in selection bias and possibly led to an over-representation of healthy subjects among the controls. Indeed our hysterectomy rate among controls was ~5% lower than expected, but as there are no obvious links between hysterectomy and inflammation that we have not considered, we do not believe that these small differences would have affected the present results. A healthy control bias would most likely influence the analyses of medical conditions, specifically sexually transmitted infections (STIs). For example, if participating controls were less likely to have had an STI this could bias risk estimates for STIs upwards. While we saw a positive association between herpes infection and ovarian cancer risk, there was no association with other STIs suggesting that our ORs are not systematically biased. Overall, a small number of participants reported STIs and it is possible that STIs were underreported because of possible asymptomatic infection or because of the negative connotations associated with having an STI. It is also possible that controls would be more likely to underreport STIs than cases therefore potentially biasing the risk estimates upwards. Another general limitation was that analyses of medical conditions were based entirely on self-reported medical history and as a result the accuracy of these reports could not be confirmed, although self-reports of these miscellaneous conditions are unlikely to be influenced greatly by case/control status.

In summary, most factors that could potentially cause ovarian inflammation (such as PID, HPV infection, and postpubertal mumps) were not associated with a significant elevation in ovarian cancer risk in our study. In addition, the expected corollary, an inverse association with regular use of anti-inflammatory medications, was not observed. While some subtype-specific associations were observed, these were not strong and showed no coherent pattern of association within or across subtypes, aside from the well-recognised increase in risk of endometrioid and clear cell cancers among women with endometriosis. The elevation in ovarian cancer risk associated with use of talc in the perineal region that we and others have observed has been regarded as the main evidence supporting an inflammatory mechanism in the development of epithelial ovarian cancer. However, experimental evidence that perineal talc use elicits an inflammatory response in the ovaries is lacking and overall we conclude that chronic inflammation does not play a major role in the development of ovarian cancer.

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References

1. Ness RB, Cottreau C. Possible role of ovarian epithelial inflammation in ovarian cancer. J Natl Cancer Inst 1999;91:1459–67.
2. Balkwill F, Mantovani A. Inflammation and cancer: back to Virchow? Lancet 2001;357:539–45.
3. Baniyash M. The inflammation-cancer linkage: a double-edged sword? Semin Cancer Biol 2006;16:1–2.
4. Cramer DW, Liberman RF, Titus-Ernstoff L, Welch WR, Greenberg ER, Baron JA, Harlow BL. Genital talc exposure and risk of ovarian cancer. Int J Cancer 1999;81:351–6.
5. Huncharek M, Geschwind JF, Kupelnick B. Perineal application of cosmetic talc and risk of invasive epithelial ovarian cancer: a meta-analysis of 11,933 subjects from sixteen observational studies. Anticancer Res 2003;23:1955–60.
6. Cook LS, Kamb ML, Weiss NS. Perineal powder exposure and the risk of ovarian cancer. Am J Epidemiol 1997;145:459–65.
7. Gertig DM, Hunter DJ, Cramer DW, Colditz GA, Speizer FE, Willett WC, Hankinson SE. Prospective study of talc use and ovarian cancer. J Natl Cancer Inst 2000;92:249–52.
8. Ness RB, Grisso JA, Cottreau C, Klapper J, Vergona R, Wheeler JE, Morgan M, Schlesselman JJ. Factors related to inflammation of the ovarian epithelium and risk of ovarian cancer. Epidemiology 2000;11:111–17.
9. McSorley MA, Alberg AJ, Allen DS, Allen NE, Brinton LA, Dorgan JF, Pollak M, Tao Y, Helzlsouer KJ. C-reactive protein concentrations and subsequent ovarian cancer risk. Obstet Gynecol 2007;109:933–41.
10. Harlow BL, Cramer DW, Bell DA, Welch WR. Perineal exposure to talc and ovarian cancer risk. Obstet Gynecol 1992;80:19–26.
11. Rohl AN, Langer AM, Selikoff IJ, Tordini A, Klimentidis R, Bowes DR, Skinner DL. Consumer talcums and powders: mineral and chemical characterization. J Toxicol Environ Health 1976;2:255–84.

12. Jenny NS, Yanez ND, Psaty BP, Kuller LH, Hirsch CH, Tracy RP. Inflammation biomarkers and near-term death in older men. Am J Epidemiol 2007;165:684–95.
13. Chang S, Risch HA. Perineal talc exposure and risk of ovarian carcinoma. Cancer 1997;79:2396–401.
14. Mills PK, Riordan DG, Cress RD, Young HA. Perineal talc exposure and epithelial ovarian cancer risk in the Central Valley of California. Int J Cancer 2004;112:458–64.
15. Fleming JS, Beaugie CR, Haviv I, Chenevix-Trench G, Tan OL. Incessant ovulation, inflammation and epithelial ovarian carcinogenesis: revisiting old hypotheses. Mol Cell Endocrinol 2006;247:4–21.
16. Henderson WJ, Hamilton TC, Baylis MS, Pierrepoint CG, Griffiths K. The demonstration of the migration of talc from the vagina and posterior uterus to the ovary in the rat. Environ Res 1986;40:247–50.
17. Heller DS, Westhoff C, Gordon RE, Katz N. The relationship between perineal cosmetic talc usage and ovarian talc particle burden. Am J Obstet Gynecol 1996;174:1507–10.
18. McCluggage WG, Allen DC. Ovarian granulomas: a report of 32 cases. J Clin Pathol 1997;50:324–7.
19. Wehner AP. Biological effects of cosmetic talc. Food Chem Toxicol 1994;32:1173–84.
20. van den Tol MP, Haverlag R, van Rossen ME, Bonthuis F, Marquet RL, Jeekel J. Glove powder promotes adhesion formation and facilitates tumour cell adhesion and growth. Br J Surg 2001;88:1258–63.
21. Parazzini F, La Vecchia C, Negri E, Moroni S, dal Pino D, Fedele L. Pelvic inflammatory disease and risk of ovarian cancer. Cancer Epidemiol Biomarkers Prev 1996;5:667–9.
22. Shu XO, Brinton LA, Gao YT, Yuan JM. Population-based case-control study of ovarian cancer in Shanghai. Cancer Res 1989;49:3670–4.
23. Risch HA, Howe GR. Pelvic inflammatory disease and the risk of epithelial ovarian cancer. Cancer Epidemiol Biomarkers Prev 1995;4: 447–51.
24. Ooi C, Dayan L. Genital herpes. An approach for general practitioners in Australia. Aust Fam Physician 2002;31:825–31.
25. Heinonen PK, Miettinen A. Laparoscopic study on the microbiology and severity of acute pelvic inflammatory disease. Eur J Obstet Gynecol Reprod Biol 1994;57:85–9.
26. Paavonen J, Teisala K, Heinonen PK, Aine R, Miettinen A, Lehtinen M, Gronroos P. Endometritis and acute salpingitis associated with Chlamydia trachomatis and herpes simplex virus type two. Obstet Gynecol 1985;65:288–91.
27. Lehtinen M, Rantala I, Teisala K, Heinonen PK, Lehtinen T, Aine R, Miettinen A, Gronroos P, Punnonen R, Leinikki P, Paavonen J. Detection of herpes simplex virus in women with acute pelvic inflammatory disease. J Infect Dis 1985;152:78–82.
28. Mai KT, Yazdi HM, Bertrand MA, LeSaux N, Cathcart LL. Bilateral primary ovarian squamous cell carcinoma associated with human papilloma virus infection and vulvar and cervical intraepithelial neoplasia. A case report with review of the literature. Am J Surg Pathol 1996;20:767–72.
29. Manolitsas TP, Lanham SA, Hitchcock A, Watson RH. Synchronous ovarian and cervical squamous intraepithelial neoplasia: an analysis of HPV status. Gynecol Oncol 1998;70:428–31.
30. Giordano G, D'Adda T, Gnetti L, Froio E, Merisio C, Melpignano M. Detection of human papillomavirus in organs of upper genital tract in women with cervical cancer. Int J Gynecol Cancer 2006;16:1601–7.
31. Ip SM, Wong LC, Xu CM, Cheung AN, Tsang PC, Ngan HY. Detection of human papillomavirus DNA in malignant lesions from Chinese women with carcinomas of the upper genital tract. Gynecol Oncol 2002;87:104–11.
32. Trottier H, Franco EL. The epidemiology of genital human papillomavirus infection. Vaccine 2006;24 (Suppl 1):S1–S15.
33. Morrison JC, Givens JR, Wiser WL, Fish SA. Mumps oophoritis: a cause of premature menopause. Fertil Steril 1975;26:655–9.
34. Ness RB. Endometriosis and ovarian cancer: thoughts on shared pathophysiology. Am J Obstet Gynecol 2003;189:280–94.
35. Brinton LA, Sakoda LC, Sherman ME, Frederiksen K, Kjaer SK, Graubard BI, Olsen JH, Mellemkjaer L. Relationship of benign gynecologic diseases to subsequent risk of ovarian and uterine tumors. Cancer Epidemiol Biomarkers Prev 2005;14:2929–35.
36. Somigliana E, Vigano P, Parazzini F, Stoppelli S, Giambattista E, Vercellini P. Association between endometriosis and cancer: a comprehensive review and a critical analysis of clinical and epidemiological evidence. Gynecol Oncol 2006;101:331–41.
37. Bonovas S, Filioussi K, Sitaras NM. Do nonsteroidal anti-inflammatory drugs affect the risk of developing ovarian cancer? A meta-analysis. Br J Clin Pharmacol 2005;60:194–203.
38. Harris RE, Beebe-Donk J, Doss H, Burr Doss D. Aspirin, ibuprofen, and other non-steroidal anti-inflammatory drugs in cancer prevention: a critical review of non-selective COX-2 blockade (review). Oncol Rep 2005;13:559–83.